

d.) Amendments to the Claims.

Please cancel claims 1, 3, 5, and 22-24, and amend claims 6, 7, 11, and 12 as follows:

Claims 1.-5. (canceled).

Claim 6. (currently amended) A method for detecting an 10,000 cfu/ml or less of actively respiring microorganisms in a sample comprising:

trapping the microorganisms of said sample on a solid filtration membrane;

~~treating the microorganisms according to the method of claim 1~~ incubating the trapped microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker;

digesting the microorganisms;

contacting primary antibodies prepared against a substituted formazan with the digested microorganisms to capture said primary antibodies;

contacting secondary antibodies prepared against the primary antibodies and conjugated with a detectable marker to captured primary antibodies; and

detecting the secondary antibodies that are bound to the captured primary antibodies.

Claim 7. (original) A method for detecting 10,000 cfu/ml or less of microorganisms ~~whose presence is amplified by the method of claim 1~~ comprising:

incubating the microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker

digesting the microorganisms by incubation with a lysozyme to form a cellular debris, wherein the viability marker is adsorbed on a surface of the cellular debris;

immobilizing primary antibodies specific for the viability marker on a solid support;

contacting the digested microorganisms with the immobilized primary antibodies thereby capturing the microorganisms; and

detecting the presence of the viability marker.

Claim 8. (original) The method of claim 7 wherein the step of detecting comprises:

contacting the captured digested microorganisms with a reporter antibody prepared from the primary antibody, the reporter antibody being conjugated to a detectable marker; and

detecting the reporter antibodies that bind to the captured digested microorganisms.

Claim 9. (original) The method of claim 7 wherein the step of detecting comprises detecting the captured viability marker by detecting a change in a physical, a chemical, an optical, or an electrical property of the solid support.

Claim 10. (original) The method of claim 7 further comprising the steps of:

incubating the viability marker with a primary antibody specific for the viability marker and conjugated to a reporter molecule, thereby forming a primary antibody-antigen-reporter molecule sandwich; and

detecting the reporter molecule.

Claim 11. (original) A method for detecting 10,000 cfu/ml or less of microorganisms according to the method of claim 1 comprising:

incubating the microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker;

digesting the microorganisms;

incubating the digested microorganisms with a primary antibody specific for the viability marker;

conjugating the primary antibody to a reporter molecule to form a reporter-primary antibody complex; and

detecting the reporter molecule.

Claim 12. (currently amended) A method for detecting an less than 10,000 cfu/ml of actively respiring microorganisms in a sample comprising:

treating the microorganisms according to the method of claim 1 incubating the actively respiring microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker;

digesting the microorganisms;

parasites
fungi
virus
bacteria

✓

Complete

contacting a primary antibody prepared against a substituted formazan with the digested microorganisms;

contacting a secondary antibody prepared against the primary antibody, the secondary antibody being conjugated to a reporter molecule; and

detecting the reporter molecule.

Claim 13. (original) The method of claim 12 further comprising the step of trapping the actively respiring microorganisms on a solid filtration membrane.

Claim 14. (original) The method of claim 12 wherein the reporter molecule comprises an enzyme, a bioluminescent protein, a radioisotope, a chemiluminescent dye, a visible dye, a latex particle, a magnetic particle or a fluorescent dye.

Claim 15. (original) The method of claim 12 wherein the sample is a clinical sample, a food sample, a cosmetic sample, a pharmaceutical sample, an industrial sample or an environmental sample.

Claim 16. (original) The method of claim 12 wherein the sample is a blood sample, a tissue sample, a tissue homogenate sample or a bodily fluid sample.

Claim 17. (original) The method of claim 12 wherein the microorganisms comprises a single species of microorganisms or a mixed population of microorganisms.

→ Claim 18. (original) The method of claim 12 wherein the sample contains less than 1,000 cfu/mL.

Claim 19. (original) The method of claim 12 wherein the detecting takes less than two hours.

Claim 20.-24. (canceled).

Please add the following as new claims 25-30:

Claim 25. (new) The method of claim 6, wherein the sample contains less than 1,000 cfu/mL.

Claim 26. (new) The method of claim 6, which takes less than two hours.

Claim 27. (new) The method of claim 7, wherein the microorganisms comprise 1,000

Appln. No. 10/020,923

New Attorney Docket No.: 54442-20002.10

cfu/mL or less.

Claim 28. (new) The method of claim 7, which takes less than two hours.

Claim 29. (new) The method of claim 11, wherein the microorganisms comprise 1,000 cfu/mL or less.

Claim 30. (new) The method of claim 11, which takes less than two hours.